Prenatal Arsenic Exposure and Birth Outcomes among a Population Residing near a Mining-Related Superfund Site

Birgit Claus Henn, Adrienne S. Ettinger, Marianne R. Hopkins, Rebecca Jim, Chitra Amarasiriwardena, David C. Christiani, Brent A. Coull, David C. Bellinger, and Robert O. Wright

Prenatal Arsenic Exposure and Birth Outcomes among a Population Residing near a Mining-Related Superfund Site

Birgit Claus Henn[1], Adrienne S. Ettinger[2], Marianne R. Hopkins[3], Rebecca Jim[4], Chitra Amarasiriwardena[5], David C. Christiani[3], Brent A. Coull[6], David C. Bellinger[3,7], and Robert O. Wright[5]

[1]Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts, USA; [2]Department of Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA; [3]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [4]Local Environmental Action Demanded (L.E.A.D.) Agency, Inc., Vinita, Oklahoma, USA; [5]Division of Environmental Health, Icahn School of Medicine at Mount Sinai, New York, New York, USA; [6]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; [7]Departments of Neurology and Psychiatry, Harvard Medical School and Boston Children's Hospital, Boston, Massachusetts, USA

Address correspondence to Birgit Claus Henn, Boston University School of Public Health, Department of Environmental Health, 715 Albany St., Boston, MA 02118 USA. Telephone: (617) 638-4653. Email: bclaus@bu.edu

Short running title: Environmental metals exposure and birth outcomes

ABSTRACT

Background: Limited epidemiologic data exist on prenatal arsenic exposure and fetal growth, particularly in the context of co-exposure to other toxic metals.

Objective: We examined whether prenatal arsenic exposure predicts birth outcomes among a rural U.S. population, while adjusting for exposure to lead and manganese.

Methods: We collected maternal and umbilical cord blood samples at delivery from 622 mother-infant pairs residing near a mining-related Superfund site in Northeast Oklahoma. Whole blood arsenic, lead and manganese were measured using inductively coupled plasma mass spectrometry. We modeled associations between arsenic concentrations and birth weight, gestational age, head circumference, and birth weight for gestational age.

Results: Median (25th to 75th percentile) maternal and umbilical cord blood metal concentrations, respectively, were: arsenic, 1.4 (1.0-2.3) and 2.4 (1.8-3.3) µg/l; lead, 0.6 (0.4-0.9) and 0.4 (0.3-0.6) µg/dl; manganese, 22.7 (18.8-29.3) and 41.7 (32.2-50.4) µg/l. We estimated negative associations between maternal blood arsenic concentrations and birth outcomes. In multivariable regression models adjusted for lead and manganese, an interquartile range increase in maternal blood arsenic was associated with -77.5 g birth weight (95% Confidence Interval: -127.8, -27.3), -0.13 (-0.27, 0.01) weeks gestation, -0.22 (-0.42, -0.03) cm head circumference, and -0.14 (-0.24, -0.04) birth weight for gestational age z-score units. Interactions between arsenic concentrations and lead or manganese were not statistically significant.

Conclusions: In a population with environmental exposure levels similar to the U.S. general population, maternal blood arsenic was negatively associated with fetal growth. Given the potential for relatively common fetal and early childhood arsenic exposures, our finding that prenatal arsenic can adversely impact birth outcomes is of considerable public health importance.

INTRODUCTION

Size at birth is an important predictor of early childhood survival and morbidity, and a predictor of chronic diseases in adulthood. Factors influencing fetal growth are numerous, and include maternal health and nutritional status. Maternal exposure to environmental toxicants, including tobacco smoke, air pollution, and metals, also predicts birth outcomes (Wigle et al. 2007).

Metals, including lead (Pb), manganese (Mn), and inorganic arsenic (As), readily cross the placenta (Concha et al. 1998; Graziano et al. 1990; Krachler et al. 1999), exposing the fetus. Prenatal exposure to these metals is associated with lower birth weight (Chen et al. 2014; Kordas et al. 2009; Quansah et al. 2015), shorter gestation (Xu et al. 2011; Yang et al. 2003) and smaller chest and head circumference (Guan et al. 2014; Rahman et al. 2009). Previous birth outcomes studies were conducted mostly in areas of unusually high arsenic or lead exposure, among populations that also may have been at increased risk of poor overall health and nutritional status (Huyck et al. 2007; Xu et al. 2012; Zheng et al. 2014). Few birth outcomes studies have examined associations with low to moderate levels of metal exposures that are consistent with background levels in many U.S. communities (Fei et al. 2013; Shi et al. 2015). Research on environmental lead exposure has demonstrated adverse effects at exposure concentrations much lower than in highly exposed populations (Xie et al. 2013). A similar phenomenon may be occurring for other common environmental contaminants, such as arsenic, for which most research has focused on high exposure levels, as was historically the case for lead. Recent reviews of the literature on arsenic exposure and birth outcomes called for additional epidemiologic studies with individual-level data to address the data gap in populations exposed to lower arsenic levels (Bloom et al. 2014; Quansah et al. 2015). Furthermore, exposures do not

4

occur in isolation and exposure to multiple metals may more accurately reflect real-world exposure scenarios (Hu et al. 2007). Metals often co-occur in the environment, especially near Superfund sites (Hu et al. 2007; Tchounwou et al. 2012), and studies have reported positive correlations between arsenic, lead, and manganese in biomarkers (Calderon et al. 2001; Delves et al. 1973; Joselow et al. 1978) and in environmental media (Zota et al. 2011). We are not aware of any study that has looked at associations between arsenic and birth outcomes, adjusting for lead and manganese. Given each metal's potential to be independently associated with birth outcomes, and the prevalence of concurrent exposures, adjustment for co-occurring metal exposures is important. Additionally, metals may act synergistically or antagonistically to affect birth outcomes; yet metal interactive effects have not previously been reported.

This study examines associations between prenatal arsenic exposure and birth outcomes, while simultaneously adjusting for lead and manganese, among mother-infant pairs residing in Northeast Oklahoma. Despite being located near a mining-related Superfund site, this area has relatively low levels of arsenic and lead in environmental media (Zota et al. 2011). We previously reported a nonlinear, inverted-U shaped association of prenatal manganese exposure with birth weight in this population (Zota et al. 2009); here, we expand this work to arsenic.

METHODS

Study Subjects

Subjects were participants in a prospective birth cohort study (Metals Assessment Targeting Community Health, "MATCH") of biologic markers of prenatal and early childhood exposure to metals, maternal psychosocial stress, and their impacts on neurodevelopment. This cohort was

enrolled in the area surrounding the Tar Creek Superfund site in Ottawa County, Oklahoma.

Details of the study including location and objectives have been described elsewhere (Ettinger et

al. 2009; Zota et al. 2009). Briefly, pregnant women were recruited during prenatal visits or at

delivery from the Integris Baptist Regional Health Center (Miami, Oklahoma), the only birthing

facility in the county. Eligibility criteria included (1) giving birth at Integris Hospital; (2)

intention to live within the study area for the next 2 years; (3) not currently enrolled in the study

with another child; and (4) English-language proficiency sufficient to participate in the informed

consent process. Eligible mothers received a detailed explanation of study procedures before

consenting to participate. The research protocol was approved by the Human Subjects

Committees of Integris Health and Harvard School of Public Health (HSPH).

Between 2002 and 2011, 1996 individuals were screened, of whom 1322 met inclusion criteria.

Of these, 713 mother-infant pairs were enrolled in MATCH. For the present study, we excluded

children with very low birth weights (i.e., <1500 g; n=1), multiple births (n=7), and if data were

missing on birth weight (n=1), gestational age (n=3), or all metal biomarker levels (n=2). Metal

biomarker data were excluded on 70 additional mother-infant pairs to avoid batch effects

inherent in different instruments due to our laboratory's purchase of a new ICP-MS instrument

near the end of the study, leaving 622 mother-infant pairs for this analysis.

Exposure Assessment

Maternal blood and umbilical cord blood samples were collected at delivery (± 12 h) and

analyzed for total arsenic concentration at the Trace Metals Laboratory at HSPH (Boston, MA).

Blood lead and manganese concentrations were also measured and considered as covariates in all

analyses. Venous whole blood was collected in trace element-free tubes [BD Vacutainer royal

blue top, with K2EDTA #368381 (Becton Dickinson, Franklin Lakes, NJ)] and shipped frozen to

the laboratory. Blood (1 ml) was digested with concentrated HNO_3 acid, followed by addition of

hydrogen peroxide and dilution with deionized water. Total arsenic, lead, and manganese

concentrations were measured with a dynamic reaction cell/inductively coupled plasma mass

spectrometer (DRC/ICP-MS; Elan 6100; PerkinElmer, Norwalk, CT) using previously published

methods and quality control procedures (Chen et al. 1999; Ettinger et al. 2009). Average

recovery of quality control standards was 75 to 110%. The limit of detection (LOD) was 0.02

µg/dl for blood arsenic, lead, and manganese. Two (0.3%) arsenic and three (0.5%) lead

measurements in cord blood were below the LOD, for which we assigned a value of half the

LOD.

Birth Outcomes and Covariates

Data on the four birth outcomes examined in this study (birth weight, birth length, head

circumference, and gestational age at birth) were abstracted from medical records by study staff

who were blind to exposure biomarker levels. Birth weight, length and head circumference were

measured by delivery room staff using standard clinical procedures. Gestational age at birth was

based on clinical assessment using data from the last menstrual period, the first accurate

ultrasound examination during the first trimester, and clinical examination (ACOG 2014).

Information on maternal pregnancy health and prenatal care such as glucose challenge test

results was obtained from medical records. Interviewer-administered questionnaires were used to

collect information on potential sources of metals exposure, pre-pregnancy weight and

demographic and social characteristics such as mother's birth date, marital status, race/ethnicity, smoking status, and prenatal vitamin use.

Statistical Analysis

Univariate and bivariate summary statistics and distributional plots were generated for all variables. Spearman correlations were calculated among exposure biomarkers. Distributions of metal concentrations were positively skewed; therefore, we used natural log-transformed metals in all models.

Associations between arsenic and four birth outcomes were estimated: birth weight (g), gestational age (weeks), head circumference (cm) and birth weight for gestational age (z-score). Weight for gestational age, an estimate of fetal growth, was calculated based on the median birth weight for each completed week of gestation for a 1999-2000 U.S. natality data set (Oken et al. 2003). Weight for gestational age was then converted to a normal z value, where each unit represents the distance of birth weight from population median for a given gestational age scaled by population standard deviation (Oken et al. 2004).

To estimate associations between arsenic and birth outcomes, we used semiparametric regression that allows for possible nonlinearity in associations between covariates and birth outcomes. All models included arsenic, lead, and manganese (lead and manganese centered at mean of $\log_e$ distribution) as well as an *a priori* set of covariates consistently associated with fetal growth parameters in the literature: maternal age (years) at child's birth, infant sex, race/ethnicity (White, Native American, Other [African American or black, Asian, Native Hawaiian, Pacific Islander]), primiparity and maternal smoking during pregnancy. Additional maternal

8

characteristics considered as potential confounders were: pre-pregnancy body mass index (BMI; kg/m^2), gestational weight gain (kg), blood (plasma) glucose (mg/dl) measured one hour after a 50g oral glucose challenge between 24 and 28 weeks gestation (as part of routine prenatal care), hemoglobin (g/dl) at delivery, education ($\geq$12th vs. <12th grade), marital status (married/living with partner vs. not), any smokers in the home during pregnancy and prenatal vitamin use. Inclusion of covariates in final multivariable models was based on 1) covariate associations with biomarkers and birth outcomes in bivariate models (α=0.1), 2) model fit (partial F-test at α=0.1) and 3) change in arsenic effect estimates (>10%). All final multivariable models were adjusted for blood lead and manganese, infant sex, maternal age at child's birth, race/ethnicity, parity, smoking during pregnancy, education, prenatal vitamin use, and hemoglobin. We modeled lead and manganese as smoothed terms using generalized additive models (GAMs) with penalized splines, given previous evidence of nonlinear associations with birth weight (Zhu et al. 2010; Zota et al. 2009). Maternal age at child's birth was modeled as a smoothed term because visual assessment of exploratory smoothed plots suggested deviations from linearity. We performed complete case analyses and assumed data were missing at random.

To obtain effect estimates for arsenic, we modeled arsenic concentrations as: (1) quartiles, with the lowest quartile as the referent group and (2) continuous log$_e$-transformed concentrations to compare the 75th to 25th percentile (interquartile range increase). Birth outcomes were modeled as continuous variables. To assess maternal and cord blood arsenic as two different proxies of prenatal exposure and to compare their ability to predict birth outcomes, we additionally fit models including both maternal and cord blood arsenic simultaneously. A p-value <0.05 was considered statistically significant. We explored arsenic interactions with lead and manganese on

9

birth outcomes by including cross-product terms of $\log_e$-transformed metals in the models (i.e.,

arsenic*lead, arsenic*manganese). Interaction cross-product terms were considered to be

statistically significant at p<0.1.

Due to our previous finding in this cohort of a positive association between maternal blood

arsenic and impaired glucose tolerance during pregnancy (Ettinger et al. 2009), we evaluated

impaired glucose tolerance as a potential confounder and effect modifier of arsenic-birth

outcomes associations. To evaluate potential confounding, regression models were adjusted for

impaired glucose tolerance, defined as blood glucose level ≥140 mg/dl (vs. <140 mg/dl)

measured one hour after a 50g glucose challenge, which is used as standard screening criteria to

identify women who should receive further testing for gestational diabetes (Vandorsten et al.

2013).. To evaluate interaction, two approaches were used: (1) models were stratified by

impaired glucose tolerance status and (2) models included a cross-product term between arsenic

and impaired glucose tolerance.

Arsenic may influence fetal development in a sex-dependent manner (Kippler et al. 2012; Xu et

al. 2011). Therefore, we assessed sex differences by stratifying analyses and by evaluating a sex-

arsenic cross-product term.

Sensitivity analyses assessed the robustness of the observed associations to evaluate: (1) the

extent of confounding by co-occurring metals, by excluding lead and manganese from models

but adjusting for other covariates; (2) arsenic effects on head circumference independent of

gestational length, by including gestational age at birth as a covariate; and (3) the extent of

exposure measurement error, which may be particularly important when examining biomarkers

10

measured in the same medium (Pollack et al. 2013), by weighting models with the inverse of the

arsenic measurement error (exposure) variance of the five laboratory replicates per sample

(Cochran and Carroll 1953). This inverse variance weighting approach assigns greater weight to

measurements with low uncertainties (Bevington and Robinson 2003). We used SAS version 9.4

(SAS Institute, Inc., Cary, NC, USA) and R version 3.1.2 (The R Foundation for Statistical

Computing, www.r-project.org)(R Development Core Team 2008).

RESULTS

Sociodemographic characteristics and birth outcomes are summarized in **Table 1**. Included

mother-infant pairs (n=622) were similar on most characteristics to subjects who were excluded

from analyses (n=91; **Table 1**). Compared to excluded mother-infant pairs, included pairs more

frequently reported smoking during pregnancy (p=0.03) and taking prenatal vitamins (p<0.0001).

Data were not available on certain characteristics (**Table 1**), primarily because information was

missing from medical records or participants may have chosen to omit information related to

certain family characteristics, potential sources of exposure, or psychosocial stress during

questionnaire administration by trained interviewers. Of 622 mother-infant pairs, data were not

available for four (0.6%) maternal and 13 (2%) cord blood arsenic measurements, three (0.5%)

cord blood lead and manganese measurements, and 24 (3.9%) head circumference measurements

at birth.

Metals concentrations in maternal and infant biomarkers are presented in **Table 2**. Median (25th

to 75th percentile) maternal and umbilical cord blood metal concentrations, respectively, were:

arsenic, 1.4 (0.97-2.3) and 2.4 (1.8-3.3) μg/l; lead, 0.60 (0.41-0.88) and 0.43 (0.28-0.62) μg/dl;

manganese, 22.7 (18.8-29.3) and 41.7 (32.2-50.4) µg/l. Maternal blood concentrations were

correlated with cord blood concentrations for: arsenic, r=0.35 (p<0.001); lead, r=0.75 (p<0.001);

and manganese, r=0.39 (p<0.001) (**Table 2**). In maternal blood, weak correlations with arsenic

were observed for lead (r=0.10, p=0.01) and manganese (r=0.11, p=0.01). Manganese and lead

were also weakly correlated (r=0.16, p<0.001). In cord blood, correlations with lead were

observed for arsenic (r=0.18, p<0.001) and manganese (r=0.23, p<0.001). Cord blood manganese

was significantly higher among boys than girls (geometric mean (geometric standard deviation):

boys, 41.1 (1.5); girls, 37.4 (1.5) µg/l, p=0.004). No significant sex differences were observed in

other biomarkers.

Bivariate analyses with arsenic modeled as a continuous outcome variable ($\log_e$ scale) showed

that prenatal vitamin use was associated with lower maternal (28%) and cord (15%) blood

arsenic (maternal: β = -0.33 [95% CI: -0.44, -0.23], cord: β = -0.16 [-0.27, -0.06]). Primiparous

mothers had 12% lower arsenic than multiparous women (β = -0.13 [-0.24, -0.03]). Compared to

white mothers, Native American mothers had 14% higher (β = 0.13 [0.01, 0.26]) and other

ethnicities had 17% lower (β = -0.19 [-0.38, -0.01]) arsenic levels. Maternal characteristics

associated with significantly (p<0.05) higher maternal arsenic were: higher age at delivery,

higher blood glucose challenge levels, lower hemoglobin at delivery, education ($\geq 12^{th}$ grade) and

being married/living with partner (data not shown). Fewer significant associations were observed

between cord blood arsenic and covariates.

In multivariable models, maternal blood arsenic was consistently negatively associated with all

outcomes. Adjusted effect estimates and 95% confidence intervals for all birth outcomes across

quartiles of maternal blood arsenic are presented in **Figure 1**. Compared to low exposure

(quartile 1), high maternal arsenic exposure (quartile 4) was associated with significantly lower

birth weight (β_{Q4} = -142.5 [-252.9, -32.2]), shorter gestational length (β_{Q4} = -0.41 [-0.72, -0.11]),

smaller head circumference (β_{Q4} = -0.48 [-0.91, -0.05]), and lower birth weight for gestational

age z-scores (β_{Q4} = -0.21 [-0.43, 0.01])(see **Table S1**). An interquartile range increase in arsenic

was associated with: -77.5 [-127.8, -27.3] g birth weight; -0.13 (-0.27, 0.01) weeks gestation; -

0.22 [-0.42, -0.03] cm head circumference; and -0.14 [-0.24, -0.04] weight for gestational age z-

scores (see **Table S1)**

Associations between cord blood arsenic and birth outcomes were less apparent. **Figure 2**

presents adjusted effect estimates and 95% confidence intervals across quartiles of cord blood

arsenic with all birth outcomes. Compared to low levels (quartile 1), high cord arsenic levels

(quartile 4) were associated with lower birth weight (β_{Q4} = -34.6 [-146.1, 76.9]) and birth weight

for gestational age z-scores (β_{Q4} = -0.09 [-0.31, 0.13]), but these associations were not

statistically significant (see **Table S1)**. There were positive associations between cord blood

arsenic (quartiles) and gestational age, though the association was only statistically significant in

quartile 2 (vs. quartile 1, β_{Q2} = 0.30 [0.00, 0.59]). There was no difference in fit between models

with cord arsenic parameterized as quartiles and as continuous $\log_e$-transformed concentrations

(likelihood ratio tests: p>0.05 for all birth outcomes) and no significant association between

continuous cord arsenic and birth outcomes (see **Table S1**).

In models including both maternal and cord blood arsenic, effect estimates for cord arsenic were

similar while estimates for maternal arsenic were attenuated (data not shown), but remained

negative and statistically significant for most relationships (e.g., -58.9 [-114.5, -3.2] g birth

weight for interquartile range increase in maternal arsenic, -0.38 [-0.71, -0.06] weeks gestation

13

for quartile 4 vs. quartile 1, -0.23 [-0.45, -0.01] cm head circumference for interquartile range

increase). No significant interactions were observed between maternal or cord blood arsenic with

lead or manganese (see **Table S2**). When we included a cross-product term between arsenic and

sex, the interaction terms were not significant for maternal or cord blood arsenic with any birth

outcomes (e.g., birth weight: maternal As*sex, p=0.6; data not shown).

Given our previous report in which blood arsenic was associated with impaired glucose tolerance

during pregnancy (Ettinger et al. 2009), we examined impaired glucose tolerance ($\geq$140 mg/dl

vs. <140 mg/dl; prevalence of impaired glucose tolerance = 12.9%) as a potential confounder

and effect modifier of arsenic-birth outcomes associations. Adjusting for impaired glucose

tolerance changed maternal and cord blood arsenic effect estimates minimally (e.g., for

interquartile range increase in maternal arsenic, with adjustment: -72.8 [-124.2, -21.4] g birth

weight; without: -77.5 [-127.8, -27.3] g; data not shown), suggesting that impaired glucose

tolerance status is not a strong confounder of the arsenic-birth outcomes associations. In these

models, impaired glucose tolerance was positively associated with weight for gestational age (β

= 0.25 [0.01, 0.49] z-scores); no other associations were observed between impaired glucose

tolerance and birth outcomes (data not shown). Glucose tolerance status may also be a causal

intermediate between arsenic and birth outcomes. Maternal arsenic was positively associated

with impaired glucose tolerance (p=0.007); however, the small changes in arsenic estimates

when conditioning on glucose intolerance suggest that impaired glucose tolerance may only be a

weak, partial mediator of the arsenic-birth outcomes associations. To evaluate effect

modification, we stratified by impaired glucose tolerance status. Compared to women with

normal post-challenge glucose levels, those with high glucose had steeper slopes for arsenic with

14

all birth outcomes, though confidence intervals were wider (**Table 3**). In models including cross-product terms between arsenic and impaired glucose tolerance status, there was a significant negative interaction between maternal arsenic and glucose tolerance status on gestational age (e.g., p=0.04 for continuous arsenic-impaired glucose tolerance cross-product term; **Table 3**), suggesting that high arsenic exposure is associated with accelerated shortened gestational length in the presence of abnormal glucose tolerance.

We fit models without lead and manganese to examine the extent of confounding by co-occurring metals (data not shown). Arsenic effect estimates were similar to those from models with lead and manganese. The main difference was found for birth weight with slightly attenuated arsenic estimates (e.g., for interquartile range increase in maternal arsenic, without lead and manganese: -68.8 [-118.5, -19.0] g; with: -77.5 [-127.8, -27.3] g). When we additionally adjusted for gestational age in models of head circumference, associations for maternal arsenic weakened (for interquartile range increase in maternal arsenic, with gestational age: -0.16 [-0.35, 0.03] cm; without: -0.22 [-0.42, -0.03] cm). In models accounting for measurement error (weighted by the inverse of arsenic measurement error variance), arsenic effect estimates changed little (see **Table S3**).

DISCUSSION

In this U.S. population, arsenic concentrations in maternal and cord blood were lower relative to studies conducted in Asia and South America (Ahmed et al. 2012; Concha et al. 1998; Guan et al. 2012), where naturally-occurring geologic factors likely caused increased arsenic exposures from drinking water. Compared to other populations with no known source of high arsenic

15

exposure in the U.S. (Sanders et al. 2012) and Europe (Remy et al. 2014), blood arsenic levels near Tar Creek are slightly higher, perhaps reflecting exposure from nearby mining waste: GM, cord blood = 0.56 μg/l (Remy et al. 2014), third trimester maternal blood = 0.44 μg/l (Sanders et al. 2012) vs. Tar Creek cord blood = 2.3 μg/l, maternal blood = 1.5 μg/l. Even with lower-level exposures, we observed decreases in birth weight, fetal growth, gestational length, and head circumference with increasing prenatal arsenic exposure while adjusting for lead and manganese. This is among the first studies of metals and birth outcomes to consider metal co-exposures as potential confounders and effect modifiers. Simultaneous exposure to multiple metals is a realistic exposure scenario, particularly in areas such as this Superfund site (Hu et al. 2007). Based on our results, for an interquartile range increase in maternal blood arsenic (1.3 μg/l), birth weight is estimated to decrease by 77.5 [-127.8, -27.3] g, which is comparable in magnitude to estimated effects of prenatal secondhand tobacco smoke on infant birth weight among nonsmoking mothers (25 to 90 g decrease based on a review of studies published through 1998)(Misra and Nguyen 1999).

Our study is consistent with much of the previous research on arsenic and birth outcomes. Prior studies have measured arsenic in maternal and cord blood (Guan et al. 2012; Remy et al. 2014; Xu et al. 2011), urine (Fei et al. 2013; Rahman et al. 2009), hair (Huyck et al. 2007), and drinking water (Hopenhayn et al. 2003; Yang et al. 2003). Despite the use of different exposure measures, inverse associations have consistently been reported with fetal growth parameters. In New Hampshire, where private wells are common, maternal urinary arsenic was inversely associated with birth weight (Fei et al. 2013) and town-level modeled groundwater arsenic was associated with town-level term low birth weight in a geospatial analysis (Shi et al. 2015). In

16

Bangladesh, a steeper negative dose response curve for arsenic with birth outcomes was

estimated at the lower exposure range (<100 μg/l in urine) compared to higher exposures

(Rahman et al. 2009). Associations with lower-level exposures were also observed in a Belgian

population (Remy et al. 2014), where blood arsenic was even lower than in our study (cord blood

GM = 0.56 μg/l).

Oxidative stress, inflammation, and placental insufficiency may be mechanisms that account for

these findings. Arsenic is a pro-oxidant (Flora 2011) and arsenic exposure has been associated

with increased levels of oxidative stress biomarkers in the placenta and in pregnant women

(Ahmed et al. 2011; Engstrom et al. 2010). Prenatal arsenic has also been associated with

increased expression of genes involved in inflammation, apoptosis and stress response in cord

blood (Fry et al. 2007), which could contribute adversely to fetal growth. Placental insufficiency,

either via oxidative stress or other mechanisms such as epigenetic modifications, may link

prenatal arsenic exposure to reduced fetal growth. Higher arsenic concentrations and reduced

birth weight were associated with increased expression of a gene whose protein product

scavenges vascular endothelial growth factor and plays a role in inhibiting placental angiogenesis

(Remy et al. 2014). Inhibited placental angiogenesis can impair nutrition and restrict growth.

Arsenic may also modulate expression of two arsenic-related genes (AQP9, ENPP2) in the

placenta that are involved in arsenic transport and regulation of angiogenesis, respectively (Fei et

al. 2013). The hormone leptin, which regulates appetite and metabolism, may also be involved:

positive associations have recently been reported between prenatal arsenic and cord blood leptin

(Gossai et al. 2015), and cord blood leptin was negatively associated with length and head

circumference at birth, and with lower weight gain from birth to 4 months, in 136-197 infants in

the ALSPAC cohort (Ong et al. 1999), as well as with lower BMI z-score, lower height-for-age

z-score, and shorter leg length in 3-year old children in the Project Viva cohort (Mantzoros et al.

2009). Arsenic exposure, even at relatively low to moderate levels, has been associated with

evidence of epigenetic effects including global DNA hypomethylation and gene-specific

hypermethylation, which may also be involved in its toxic mechanism of action (Bailey and Fry

2014; Intarasunanont et al. 2012).

In our data, inverse associations between arsenic and birth outcomes appeared stronger among

women with impaired glucose tolerance compared to women without, though effect estimates

were less precise due to the small number of subjects with impaired glucose tolerance. In the

presence of impaired maternal glucose tolerance, arsenic may limit fetal growth more than

among women with normal glucose metabolism. It is known that maternal hyperglycemia

increases fetal insulin, which stimulates growth, and therefore, offspring of mothers with

gestational diabetes have higher birth weights (Silverman et al. 1991). Further, there is evidence

linking elevated arsenic exposure with increased risk of impaired glucose tolerance during

pregnancy (Ettinger et al. 2009) and Type II diabetes (Navas-Acien et al. 2008). Thus, one might

expect impaired maternal glucose metabolism to reduce, rather than increase, the magnitude of

the inverse association between arsenic and birth weight. On the other hand, low birth weight has

been associated with subsequent risk of Type II diabetes (Harder et al. 2009), suggesting a link

between insulin resistance and fetal thinness. Others have proposed that insulin resistance in the

fetus, which may be genetically determined and/or influenced by maternal hyperglycemia, could

impair angiogenesis (Hattersley and Tooke 1999), thereby reducing fetal growth. It is also

possible that prenatal arsenic exposure lowers infant birth weight via a mechanism that acts

18

independently of glucose metabolism, such as oxidative stress, overriding any effect of insulin to increase body weight. Both indirect effects on the fetus via maternal impaired glucose tolerance and direct effects via fetal insulin resistance could confer metabolic dysfunction on the fetus and increase later risk of Type II diabetes.

There are limitations to this study. The use of blood to characterize arsenic exposure may be imperfect due to the short half life in blood (Pomroy et al. 1980); therefore, exposure misclassification is possible. However, with continuous and steady exposure, steady-state concentrations of blood arsenic may be reached (National Research Council 1999). We measured total arsenic, which includes inorganic and organic species that may vary in their toxicity. Lack of speciated data could result in exposure misclassification. However, new research suggests that organoarsenicals, previously thought to be non-toxic, as well as inorganic species, may be transformed endogenously in humans (Molin et al. 2015); this adds to the total potential toxic arsenic burden that would be captured by measurements of total arsenic. Further, organoarsenicals likely represent a small fraction of total arsenic exposure because consumption of seafood, a primary source of organic arsenic exposure, is low in this population (62% of mothers reported never consuming a 3-5 oz serving of fish during pregnancy). The small number of women with impaired glucose tolerance limits our power to examine glucose-arsenic interactions in further depth. Unmeasured and residual confounding by smoking, secondhand smoke, or maternal diet/nutritional status is possible. Residual confounding by manganese exposure is also possible given that blood manganese is under tight regulatory control and may not be sensitive enough to accurately represent subtle differences in exposure. Finally, we found

19

no statistically significant interactions between arsenic and lead or manganese, although joint

effects of these three metals are possible and should be evaluated in future studies.

There are several strengths including that this study is among the first to examine associations

between low-level metal exposures and birth outcomes in the U.S. The availability of biomarker

data on arsenic, lead, and manganese made it possible to adjust for important co-exposures,

which few other studies have done. We evaluated both maternal and umbilical cord blood as

biomarkers of prenatal exposure. Our finding that maternal arsenic was more strongly associated

with birth outcomes than infant arsenic is consistent with the two other published studies with

both biomarkers available (Guan et al. 2012; Xu et al. 2011), suggesting that maternal blood

arsenic may be a more informative biomarker.

Exposure to arsenic remains a significant public health concern, especially in light of recent

evidence that common consumer products contribute to exposures among pregnant women and

children. Elevated arsenic concentrations have been measured in rice, rice products, fruit juices

and infant formulas (Gilbert-Diamond et al. 2011; Jackson et al. 2012; Wilson et al. 2012).

Given the potential for relatively common fetal and early childhood exposures to arsenic, our

finding that prenatal arsenic, even at low to moderate levels, can adversely impact birth

outcomes is of considerable public health importance.

CONCLUSIONS

Inverse associations between prenatal arsenic exposures and birth outcomes were observed while

controlling for co-exposure to lead and manganese. This is one of the first reports of

environmental arsenic associations with birth outcomes in a healthy U.S. population. These

20

findings have wide-reaching implications as they are more generalizable to the U.S. population

than studies conducted in highly-exposed populations.

REFERENCES

ACOG. 2014. American college of obstetricians and gynecologists. Method for estimating due date. Obstetrics and gynecology 124:863-866.

Ahmed S, Mahabbat-e Khoda S, Rekha RS, Gardner RM, Ameer SS, Moore S, et al. 2011. Arsenic-associated oxidative stress, inflammation, and immune disruption in human placenta and cord blood. Environ Health Perspect 119:258-264.

Ahmed S, Ahsan KB, Kippler M, Mily A, Wagatsuma Y, Hoque AM, et al. 2012. In utero arsenic exposure is associated with impaired thymic function in newborns possibly via oxidative stress and apoptosis. Toxicol Sci 129:305-314.

Bailey KA, Fry RC. 2014. Arsenic-associated changes to the epigenome: What are the functional consequences? Current environmental health reports 1:22-34.

Bevington PR, Robinson DK. 2003. Data reduction and error analysis for the physical sciences 3rd ed. New York:McGraw Hill.

Bloom MS, Surdu S, Neamtiu IA, Gurzau ES. 2014. Maternal arsenic exposure and birth outcomes: A comprehensive review of the epidemiologic literature focused on drinking water. Int J Hyg Environ Health 217:709-719.

Calderon J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, et al. 2001. Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res 85:69-76.

Chen KL, Amarasiriwardena CJ, Christiani DC. 1999. Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. Biol Trace Elem Res 67:109-125.

Chen L, Ding G, Gao Y, Wang P, Shi R, Huang H, et al. 2014. Manganese concentrations in maternal-infant blood and birth weight. Environ Sci Pollut Res Int 21:6170-6175.

Cochran WG, Carroll SP. 1953. A sampling investigation of the efficiency of weighting inversely as the estimated variance. Biometrics 9:447-459.

Concha G, Vogler G, Lezcano D, Nermell B, Vahter M. 1998. Exposure to inorganic arsenic metabolites during early human development. Toxicol Sci 44:185-190.

Delves HT, Clayton BE, Bicknell J. 1973. Concentration of trace metals in the blood of children. Br J Prev Soc Med 27:100-107.

Engstrom KS, Vahter M, Johansson G, Lindh CH, Teichert F, Singh R, et al. 2010. Chronic exposure to cadmium and arsenic strongly influences concentrations of 8-oxo-7,8-dihydro-2'-deoxyguanosine in urine. Free Radic Biol Med 48:1211-1217.

Ettinger AS, Zota AR, Amarasiriwardena CJ, Hopkins MR, Schwartz J, Hu H, et al. 2009. Maternal arsenic exposure and impaired glucose tolerance during pregnancy. Environ Health Perspect 117:1059-1064.

Fei DL, Koestler DC, Li Z, Giambelli C, Sanchez-Mejias A, Gosse JA, et al. 2013. Association between in utero arsenic exposure, placental gene expression, and infant birth weight: A US birth cohort study. Environ Health 12:58.

Flora SJ. 2011. Arsenic-induced oxidative stress and its reversibility. Free Radic Biol Med 51:257-281.

Fry RC, Navasumrit P, Valiathan C, Svensson JP, Hogan BJ, Luo M, et al. 2007. Activation of inflammation/NF-kappaB signaling in infants born to arsenic-exposed mothers. PLoS Genet 3:e207.

Gilbert-Diamond D, Cottingham KL, Gruber JF, Punshon T, Sayarath V, Gandolfi AJ, et al. 2011. Rice consumption contributes to arsenic exposure in US women. Proc Natl Acad Sci U S A 108:20656-20660.

Gossai A, Lesseur C, Farzan S, Marsit C, Karagas MR, Gilbert-Diamond D. 2015. Association between maternal urinary arsenic species and infant cord blood leptin levels in a New Hampshire pregnancy cohort. Environ Res 136:180-186.

Graziano JH, Popovac D, Factor-Litvak P, Shrout P, Kline J, Murphy MJ, et al. 1990. Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. Environ Health Perspect 89:95-100.

Guan H, Piao F, Zhang X, Li X, Li Q, Xu L, et al. 2012. Prenatal exposure to arsenic and its effects on fetal development in the general population of Dalian. Biol Trace Elem Res 149:10-15.

Guan H, Wang M, Li X, Piao F, Li Q, Xu L, et al. 2014. Manganese concentrations in maternal and umbilical cord blood: Related to birth size and environmental factors. Eur J Public Health 24:150-157.

Harder T, Roepke K, Diller N, Stechling Y, Dudenhausen JW, Plagemann A. 2009. Birth weight, early weight gain, and subsequent risk of type 1 diabetes: Systematic review and meta-analysis. Am J Epidemiol 169:1428-1436.

Hattersley AT, Tooke JE. 1999. The fetal insulin hypothesis: An alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789-1792.

Hopenhayn C, Ferreccio C, Browning SR, Huang B, Peralta C, Gibb H, et al. 2003. Arsenic exposure from drinking water and birth weight. Epidemiology 14:593-602.

Hu H, Shine J, Wright RO. 2007. The challenge posed to children's health by mixtures of toxic waste: The Tar Creek Superfund site as a case-study. Pediatric clinics of North America 54:155-175, x.

Huyck KL, Kile ML, Mahiuddin G, Quamruzzaman Q, Rahman M, Breton CV, et al. 2007. Maternal arsenic exposure associated with low birth weight in Bangladesh. J Occup Environ Med 49:1097-1104.

Intarasunanont P, Navasumrit P, Waraprasit S, Chaisatra K, Suk WA, Mahidol C, et al. 2012. Effects of arsenic exposure on DNA methylation in cord blood samples from newborn babies and in a human lymphoblast cell line. Environ Health 11:31.

Jackson BP, Taylor VF, Karagas MR, Punshon T, Cottingham KL. 2012. Arsenic, organic foods, and brown rice syrup. Environ Health Perspect 120:623-626.

Joselow MM, Tobias E, Koehler R, Coleman S, Bogden J, Gause D. 1978. Manganese pollution in the city environment and its relationship to traffic density. Am J Public Health 68:557-560.

Kippler M, Wagatsuma Y, Rahman A, Nermell B, Persson LA, Raqib R, et al. 2012. Environmental exposure to arsenic and cadmium during pregnancy and fetal size: A longitudinal study in rural Bangladesh. Reprod Toxicol 34:504-511.

Kordas K, Ettinger AS, Lamadrid-Figueroa H, Tellez-Rojo MM, Hernandez-Avila M, Hu H, et al. 2009. Methylenetetrahydrofolate reductase (MTHFR) C677T, A1298C and G1793A genotypes, and the relationship between maternal folate intake, tibia lead and infant size at birth. Br J Nutr 102:907-914.

Krachler M, Rossipal E, Micetic-Turk D. 1999. Trace element transfer from the mother to the newborn--investigations on triplets of colostrum, maternal and umbilical cord sera. Eur J Clin Nutr 53:486-494.

Mantzoros CS, Rifas-Shiman SL, Williams CJ, Fargnoli JL, Kelesidis T, Gillman MW. 2009. Cord blood leptin and adiponectin as predictors of adiposity in children at 3 years of age: A prospective cohort study. Pediatrics 123:682-689.

Misra DP, Nguyen RH. 1999. Environmental tobacco smoke and low birth weight: A hazard in the workplace? Environ Health Perspect 107 Suppl 6:897-904.

Molin M, Ulven SM, Meltzer HM, Alexander J. 2015. Arsenic in the human food chain, biotransformation and toxicology - Review focusing on seafood arsenic. J Trace Elem Med Biol 31:249-259.

US National Research Council, NRC. 1999. Subcommittee on arsenic in drinking water. Washington, DC.

Navas-Acien A, Silbergeld EK, Pastor-Barriuso R, Guallar E. 2008. Arsenic exposure and prevalence of type 2 diabetes in US adults. JAMA 300:814-822.

Oken E, Kleinman KP, Rich-Edwards J, Gillman MW. 2003. A nearly continuous measure of birth weight for gestational age using a United States national reference. BMC Pediatr 3:6.

Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW, Gillman MW. 2004. Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: Results from a US pregnancy cohort. Am J Epidemiol 160:774-783.

Ong KK, Ahmed ML, Sherriff A, Woods KA, Watts A, Golding J, et al. 1999. Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. ALSPAC study team. Avon longitudinal study of pregnancy and childhood. J Clin Endocrinol Metab 84:1145-1148.

Pollack AZ, Perkins NJ, Mumford SL, Ye A, Schisterman EF. 2013. Correlated biomarker measurement error: An important threat to inference in environmental epidemiology. Am J Epidemiol 177:84-92.

Pomroy C, Charbonneau SM, McCullough RS, Tam GK. 1980. Human retention studies with 74As. Toxicol Appl Pharmacol 53:550-556.

Quansah R, Armah FA, Essumang DK, Luginaah I, Clarke E, Marfoh K, et al. 2015. Association of arsenic with adverse pregnancy outcomes/infant mortality: A systematic review and meta-analysis. Environ Health Perspect 123:412-421.

R Development Core Team. 2008. R: A language and environment for statistical computing. Vienna, Austria:R Foundation for Statistical Computing.

Rahman A, Vahter M, Smith AH, Nermell B, Yunus M, El Arifeen S, et al. 2009. Arsenic exposure during pregnancy and size at birth: A prospective cohort study in Bangladesh. Am J Epidemiol 169:304-312.

Remy S, Govarts E, Bruckers L, Paulussen M, Wens B, Hond ED, et al. 2014. Expression of the sFLT1 gene in cord blood cells is associated to maternal arsenic exposure and decreased birth weight. PLoS One 9:e92677.

Sanders AP, Flood K, Chiang S, Herring AH, Wolf L, Fry RC. 2012. Towards prenatal biomonitoring in North Carolina: Assessing arsenic, cadmium, mercury, and lead levels in pregnant women. PLoS One 7:e31354.

Shi X, Ayotte JD, Onda A, Miller S, Rees J, Gilbert-Diamond D, et al. 2015. Geospatial association between adverse birth outcomes and arsenic in groundwater in New Hampshire, USA. Environ Geochem Health 37:333-351.

Silverman BL, Rizzo T, Green OC, Cho NH, Winter RJ, Ogata ES, et al. 1991. Long-term prospective evaluation of offspring of diabetic mothers. Diabetes 40 Suppl 2:121-125.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. 2012. Heavy metal toxicity and the environment. EXS 101:133-164.

Vandorsten JP, Dodson WC, Espeland MA, Grobman WA, Guise JM, Mercer BM, et al. 2013. NIH consensus development conference: Diagnosing gestational diabetes mellitus. NIH consensus and state-of-the-science statements 29:1-31.

Wigle DT, Arbuckle TE, Walker M, Wade MG, Liu S, Krewski D. 2007. Environmental hazards: Evidence for effects on child health. J Toxicol Environ Health B Crit Rev 10:3-39.

Wilson D, Hooper C, Shi X. 2012. Arsenic and lead in juice: Apple, citrus, and apple-base. J Environ Health 75:14-20; quiz 44.

Xie X, Ding G, Cui C, Chen L, Gao Y, Zhou Y, et al. 2013. The effects of low-level prenatal lead exposure on birth outcomes. Environ Pollut 175:30-34.

Xu L, Yokoyama K, Tian Y, Piao FY, Kitamura F, Kida H, et al. 2011. Decrease in birth weight and gestational age by arsenic among the newborn in Shanghai, China. Nihon Koshu Eisei Zasshi 58:89-95.

Xu X, Yang H, Chen A, Zhou Y, Wu K, Liu J, et al. 2012. Birth outcomes related to informal e-waste recycling in Guiyu, China. Reprod Toxicol 33:94-98.

Yang CY, Chang CC, Tsai SS, Chuang HY, Ho CK, Wu TN. 2003. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. Environ Res 91:29-34.

Zheng G, Zhong H, Guo Z, Wu Z, Zhang H, Wang C, et al. 2014. Levels of heavy metals and trace elements in umbilical cord blood and the risk of adverse pregnancy outcomes: A population-based study. Biol Trace Elem Res 160:437-444.

Zhu M, Fitzgerald EF, Gelberg KH, Lin S, Druschel CM. 2010. Maternal low-level lead exposure and fetal growth. Environ Health Perspect 118:1471-1475.

Zota AR, Ettinger AS, Bouchard M, Amarasiriwardena CJ, Schwartz J, Hu H, et al. 2009. Maternal blood manganese levels and infant birth weight. Epidemiology 20:367-373.

Zota AR, Schaider LA, Ettinger AS, Wright RO, Shine JP, Spengler JD. 2011. Metal sources and exposures in the homes of young children living near a mining-impacted Superfund site. J Expo Sci Environ Epidemiol 21:495-505.

Table 1. Characteristics of mother-infant pairs

Characteristic	Included participants (n=622)[a]	Excluded participants (n=91)[b]
	n (%) or mean ± SD	n (%) or mean ± SD
Maternal		
Age at delivery (years)	24.5 ± 5.5	24.5 ± 5.2
Pre-pregnancy BMI (kg/m^2)	26.9 ± 6.4	28.8 ± 7.0
Weight gain during pregnancy (kg)	13.1 ± 7.2	13.6 ± 7.7
Marital status		
Married or living with partner	391 (64.1%)	64 (73.6%)
Never married/separated/divorced	219 (35.9%)	23 (26.4%)
Race/Ethnicity		
White	408 (67.2%)	58 (67.4%)
Native American	143 (23.6%)	24 (27.9%)
Other (including Hispanic)	56 (9.2%)	4 (4.7%)
Education		
<12th grade	159 (25.6%)	25 (28.1%)
≥12th grade	462 (74.4%)	64 (71.9%)
Annual household income		
<$20K	188 (47.5%)	45 (60.0%)
$20K - $40K	134 (33.8%)	19 (25.3%)
$40K - $70K	61 (15.4%)	7 (9.3%)
>$70K	13 (3.3%)	4 (5.3%)
Impaired glucose tolerance, 24-28 weeks gestation		
< 140 mg/dl	508 (87.1%)	65 (84.4%)
≥ 140 mg/dl	75 (12.9%)	12 (15.6%)
Primiparous		
Yes	243 (39.1%)	32 (35.6%)
No	378 (60.9%)	58 (64.4%)
Smoked during pregnancy*		
Yes	224 (36.0%)	22 (24.4%)
No	398 (64.0%)	68 (75.6%)
Any smokers in household		
Yes	134 (38.0%)	32 (40.5%)
No	219 (62.0%)	47 (59.5%)
Prenatal vitamin use*		
Yes	397 (63.8%)	32 (35.2%)
No	225 (36.2%)	59 (64.8%)
Anemia at delivery[c]		
Yes	156 (25.3%)	19 (22.4%)
No	460 (74.7%)	66 (77.6%)

Hemoglobin at delivery	11.8 ± 1.4	11.7 (1.2)
Infant		
Birth weight (g)	3370.2 ± 474.5	3245.6 ± 462.3
Gestational age at birth (week)	39.1 ± 1.3	38.7 ± 1.9
Length at birth (cm)	50.0 ± 2.6	50.8 ± 2.4
Head circumference at birth (cm)	34.5 ± 1.8	34.1 ± 1.3
Male sex	340 (54.7%)	46 (51.7%)

a – Of included participants, data were missing for pre-pregnancy BMI (n=63), weight gain during pregnancy (n=63), marital status (n=12), race/ethnicity (n=15), education (n=1), household income (n=226), impaired glucose tolerance (n=39), primiparous (n=1), any smokers in household (n=269), anemia at delivery (n=6), hemoglobin at delivery (n=6), length at birth (n=10), and head circumference at birth (n=24).

b – Of excluded participants, data were missing for age at delivery (n=3), pre-pregnancy BMI (n=6), weight gain during pregnancy (n=5), marital status (n=4), race/ethnicity (n=5), education (n=2), household income (n=16), impaired glucose tolerance (n=14), primiparous (n=1), smoked during pregnancy (n=1), any smokers in household (n=12), anemia (n=6), hemoglobin at delivery (n=6), birth weight (n=3), gestational age at birth (n=6), length at birth (n=6), head circumference at birth (n=7), and male sex (n=2).

c – Anemia defined as hemoglobin <11.0 g/dl at delivery, which is based on definition from Centers for Disease Control (during 3rd trimester), World Health Organization (during pregnancy), and the American Congress of Obstetricians and Gynecologists (in 1st and 3rd trimesters).

* Asterisk denotes that participants differed from non-participants, p<0.05 in chi-square test.

Table 2. Maternal and infant biomarkers

| Biomarker | N | Maternal blood | | N | Umbilical cord blood | | Corr[a] |
		Median (25th to 75th percentile)	Range		Median (25th to 75th percentile)	Range	
As (μg/l)	618	1.4 (0.97 – 2.3)	0.23 – 24.1	609	2.4 (1.8 – 3.3)	<LOD[b] – 13.2	0.35*
Pb (μg/dl)	622	0.60 (0.41 – 0.88)	0.03 – 3.1	619	0.43 (0.28 – 0.62)	<LOD[b] – 3.9	0.75*
Mn (μg/l)	622	22.7 (18.8 – 29.3)	8.0 – 117.4	619	41.7 (32.2 – 50.4)	5.4 – 139.1	0.39*

a – Spearman's correlation between Maternal and infant blood. Asterisk denotes p<0.001.

b – Limit of detection is 0.02 μg/dl.

Table 3. Adjusted associations[a] of maternal blood arsenic with birth outcomes, stratified by maternal glucose tolerance status

Outcome and Exposure	n	Normal (<140 mg/dl) Estimate (95% CI)[b]	n	Impaired Glucose Tolerance (≥140 mg/dl) Estimate (95% CI)[b]	P-value for Interaction[c]
Birth weight					
As Quartile 1[d]	132	0 (reference)	11	0 (reference)	
Quartile 2	120	-21.0 (-134.2, 92.2)	13	-409.0 (-771.7, -46.3)	0.33
Quartile 3	118	-112.9 (-228.3, 2.6)	24	-183.5 (-495.3, 128.3)	0.76
Quartile 4	115	-115.3 (-235.6, 5.1)	24	-562.6 (-919.6, -205.7)	0.20
Per IQR increase in As	485	-68.6 (-123.4, -13.8)	72	-185.0 (-358.6, -11.4)	0.62
Gestational Age					
As Quartile 1	132	0 (reference)	11	0 (reference)	
Quartile 2	120	0.00 (-0.31, 0.32)	13	-0.33 (-1.4, 0.75)	0.70
Quartile 3	118	-0.12 (-0.44, 0.20)	24	-0.74 (-1.7, 0.22)	0.49
Quartile 4	115	-0.33 (-0.66, 0.00)	24	-1.7 (-2.8, -0.60)	0.06*
Per IQR increase in As	485	-0.07 (-0.22, 0.08)	72	-0.73 (-1.2, -0.23)	0.04*
Birth weight for gestational age					
As Quartile 1	132	0 (reference)	11	0 (reference)	
Quartile 2	120	-0.05 (-0.28, 0.18)	13	-0.81 (-1.5, -0.09)	0.28
Quartile 3	118	-0.20 (-0.43, 0.03)	24	-0.10 (-0.73, 0.52)	0.54
Quartile 4	115	-0.18 (-0.42, 0.07)	24	-0.70 (-1.4, 0.02)	0.47
Per IQR increase in As	485	-0.14 (-0.25, -0.03)	72	-0.21 (-0.56, 0.14)	0.84
Head circumference					
As Quartile 1	127	0 (reference)	11	0 (reference)	
Quartile 2	113	-0.02 (-0.47, 0.43)	13	-1.2 (-2.5, 0.04)	0.63
Quartile 3	113	-0.58 (-1.0, -0.13)	23	-0.75 (-1.8, 0.35)	0.67
Quartile 4	112	-0.44 (-0.91, 0.04)	24	-1.9 (-3.1, -0.65)	0.58
Per IQR increase in As	465	-0.21 (-0.43, 0.01)	71	-0.67 (-1.3, -0.08)	0.87

a – Models adjusted for maternal blood Pb and Mn (smoothed), maternal age at child's birth (smoothed), infant sex, race/ethnicity, parity, smoking during pregnancy, maternal education, prenatal vitamin use, and maternal hemoglobin at delivery.

b – Effect estimates represent change in birth outcomes for (1) arsenic quartiles 2, 3, 4 compared to quartile 1, or (2) IQR increase in arsenic (continuous $\log_e$-transformed concentrations, scaled to the IQR.

c – Interaction cross-product term between arsenic and impaired glucose tolerance. Asterisk denotes interaction p-value < 0.10.

d – Arsenic quartile 1: < 0.97 μg/l, quartile 2: ≥ 0.97 to < 1.4 μg/l, quartile 3: ≥ 1.4 to < 2.3 μg/l, quartile 4: ≥ 2.3 μg/l.

FIGURE LEGENDS

Figure 1. Effect estimates and 95% confidence intervals for quartiles of maternal blood arsenic with birth weight, gestational age, weight for gestational age, and head circumference. Generalized additive models include maternal blood lead and manganese (smoothed), maternal age at delivery (smoothed), infant sex, race/ethnicity, parity, smoking during pregnancy, maternal education, prenatal vitamin use, and maternal hemoglobin at delivery (n=596 for BW, GA, BW for GA; n=574 for HC). Dashed horizontal line represents null association.

Figure 2. Effect estimates and 95% confidence intervals for quartiles of cord blood arsenic with birth weight, gestational age, weight for gestational age, and head circumference. Generalized additive models include cord blood lead and manganese (smoothed), maternal age at delivery (smoothed), infant sex, race/ethnicity, parity, smoking during pregnancy, maternal education, prenatal vitamin use, and maternal hemoglobin at delivery (n=588 for BW, GA, BW for GA; n=565 for HC). Dashed horizontal line represents null association.
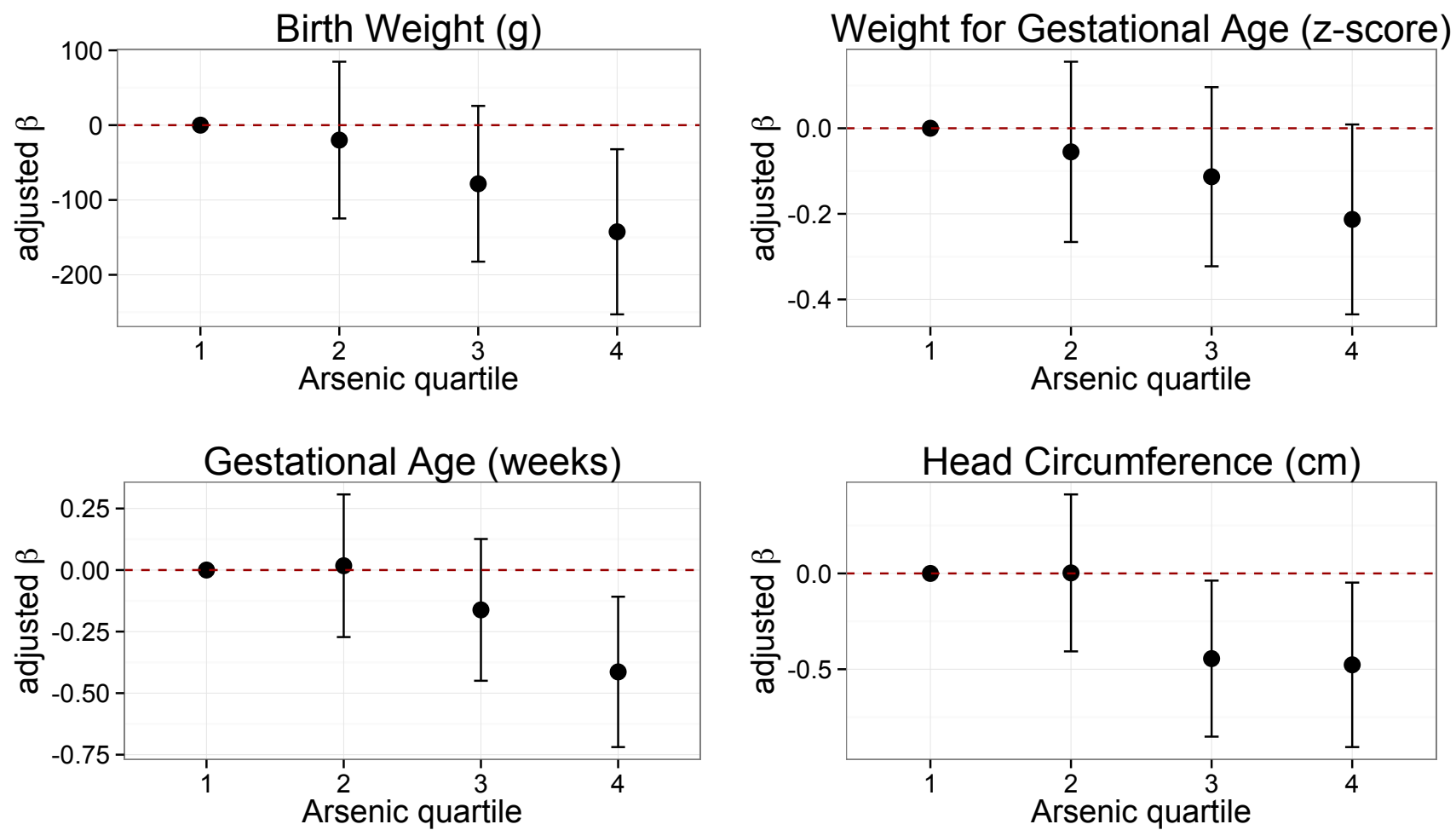
Figure 1

Figure 2